REMARKS

Reconsideration of this application is respectfully requested.

Claims 284-372 are pending in this application. Claims 284, 331, 332, 337 and 348 have been amended above by this Supplemental Response. No claims have been added or canceled. Accordingly, claims 284-372 as amended are presented for further examination on the merits.

Applicants have directed entry of this Supplemental Response under the expedited procedure provisions of Rule 1.116, notwithstanding their Request To Withdraw The Finality Of The January 6, 1998 Office Action that was concurrently filed with their July 6, 1998 Amendment.

It is believed that this Supplemental Response will further clarify or narrow the issues that were raised in the January 6, 1998 Office Action, thereby placing this application is a better position for allowance.

To help clarify or narrow the issues in this regard, Applicants have amended each of claims 284, 331, 332, 337 and 348. In each of claims 284, 331, 337 and 348, the sugar moiety in each of the Markush nucleotide members has been changed to a "monosaccharide" moiety. Further, the covalent attachment of Sig to elements in each nucleotide recited in claim 284, 331 and 337 has been limited to "such covalent attachment does not substantially interfere with double helix formation." The latter insertion adopts the Examiner's suggestion or meets his requirement for claim definiteness. See [3] under The Rejection Under 35 U.S.C. §112, second paragraph, cited and addressed on page 35 of this paper. In claim 332, the omission of the chemical structure has been corrected. That claim was originally submitted in Applicants' March 28, 1997 Amendment Under 37 C.F.R. §1.115. The missing furanose structure after line 3 of claim 332 has now been supplied above. In addition, the Markush members for elements x, y and z have been corrected by replacing them with those taken from claim 348. Evidently, a software glitch prevented them from being properly printed in the version that was ultimately submitted in Applicants' March

28, 1997 filing. Lastly, a minor typographical error, the mispelling of "pyrimidine" has been corrected in claim 332. None of the foregoing amendments to the claims is believed to constitute the insertion of new matter into the disclosure. All of the amendments to the claims are believed to be subject matter that Applicants are duly entitled to claim. Entry of the claim amendments is respectfully requested.

The Objection and Rejection Under 35 U.S.C. §112, First Paragraph

Claims 284-373 were rejected for new matter. In the January 6, 1998 Office Action (pages 3-5), the Examiner stated:

> **[1]** Consideration of the disclosure as filed has failed to reveal support for newly submitted claims 339-341. It is noted that claims 79-82 and 90 disclosure some specific linkage groups but none of the same scope as newly submitted claims 339-341. The linkages of claims 339-341, 350, 351, 353, 354, 356-358 therefore are NEW MATTER. This rejection is necessitated by amendment.
>
> **[2]** The optional template dependent or independent limitations of claims 346 and 363 have not been found as filed and are therefore NEW MATTER. This rejection is necessitated by amendment.
>
> **[3]** The specific localization of modified nucleotides as given in instant claims 365-367 has also not been found as filed and is therefore NEW MATTER. This rejection is necessitated by amendment.
>
> **[4]** The electrophoretic separating as given in instant claim 368 has also not been found as filed and is therefore NEW MATTER. This rejection is necessitated by amendment.
>
> **[5]** Consideration of the disclosure as filed has also failed to reveal written description of sequencing gel practice as now given in instant claims 329 and 348 etc. This practice therefore is NEW MATTER. This rejection is necessitated by amendment.
>
> **[6]** The following rejection is reiterated from the office action, mailed 12/28/95. Applicants argue that the content of two previous patents should overcome this rejection. In response, the rejection is based on a lack of written description "as filed" in the instant application. The content of other disclosures is moot and non-persuasive in overcoming the rejection because such other disclosures do not support what instant written basis existed as filed. There other disclosures therefore are not directed to the basis of this rejection which is a lack of support for the below summarized limitations "as originally filed". This rejection is repeated as follows and additionally applied to newly added claims that also contain the

> NEW MATTER limitations as necessitated by amendment. The limitations directed to the covalent attachment of a Sig moiety to a nucleotide base limited to positions other than C^5 of pyrimidines, C^8 of purines, or C^7 of deazapurines as presently given in claim 284 is NEW MATTER. No such negative limitations which are inclusive of numerous other base modification locations are cited in the specification. The presently pending claims dependent from claim 284 also contain the NEW MATTER due to their direct or indirect dependence from claim 284. It is noted that none of these dependent claims are limited so as to not contain said NEW MATTER limitation. Even claims such as 310 contain this limitation in that its Sig attachment limitation only limits the nucleotide (iii) selection but that the claim lacks wording such that this (iii) selection is the only labeled nucleotide type.

Applicants reiterate their previous traversal of the rejection.

In order to ensure that each and every issue in the new matter rejection is thoroughly addressed, Applicant's attorney has inserted bold bracketed numbers in the above-quoted portion from the instant Office Action. The remarks below correspond to the bracketed numbers.

[1][2][3][4][5] Regarding all of these numbered matters in the indefiniteness rejection, it is believed that the subject matter of the claims at hand is fully supported by the originally filed specification. In addition to the remarks in their just-filed July 6, 1998 Amendment, Applicants would like to submit the Declaration of James J. Donegan In Support of Adequate Description which is directed to these five points in the indefiniteness rejection. Dr. Donegan's Declaration is attached as Exhibit A. Dr. Donegan is a Senior Research Investigator at Enzo Diagnostics, Inc., the assignee of this application. At the outset, Applicants would like to point out that Dr. Donegan is well versed and experienced in the biotechnology arts, having devoted the bulk of his academic and industry career to such areas as probe hybridization technology and nucleic acid sequencing. Turning to the five numbered issues in the rejection . . .

[1] As set forth in Paragraph 13 of his Declaration (Exhibit A), Dr. Donegan points to numerous instances in the specification set forth below to support the language of claims 339-341, 350-351, 353-354 and 356-358:

Claims	Recitation	Support in Specification
339, 350 353, 356	**wherein said linkage group contains an amine**	**Page 11, 2nd full ¶** ("carbon-nitrogen bonds") **Page 11, 3rd full ¶** ("It is even more preferred that the chemical linkage group be derived from a primary amine, and have the structure –CH2–NH–, since such linkages are easily formed utilizing any of the well known amine modification reactions. Examples of preferred linkages derived from allylamine and allyl-(3-amino-2-hydroxy-1-propyl) ether groups . . . Although these linkages are preferred, others can be used, including particularly olefinic linkage arms with other modifiable functionalities such as thiol, carboxylic acid, and expoxide functionalities. **Page 98, 1st ¶** ("Accordingly, the Sig component or chemical moiety of nucleotides of this invention can be directly covalently attached . . . via a chemical linkage or linkage arm as described in U.S. patent application Ser. No. 225,223, . . . The various linkages identifed in U.S. Ser. No. 225,223 aare applicable to and useful in the preparation of the special nucleotides of this invention.") **Originally filed claims 79-80, 90, 202-203**
340, 351 354, 357	**wherein said amine comprises a primary amine**	**Page 11, 3rd full ¶** **Page 13** ("to employ olefins with primary amine functional groups, such as allylamine (AA) or allyl-(-3-amino-2-hydroxy-1-propyl) ether (NAGE)") Page 16, schema ("allylamine") Page 18, 3rd ¶ ("Examples include .") Page 98, 1st ¶, *supra*.
341, 358	**wherein said linkage group does not substantially interfere with formation of the signalling moiety or detection of the detectable signal**	**Page 7, 2nd ¶** (Fourth, the detection system should be capable of interacting with probe substituents incorporated into both single-stranded and double-stranded polynucleotides in order to be compatible with nucleic acid hybridization methodologies. To satisfy this criterion, it is preferable that the probe moiety be attached to the purine or pyrimidine through a chemical linkage or "linker arm" so that it can readily interact with antibodies, other detector proteins, or chemical reagents.") **Page 11, 2nd ¶** ("The linkage or group joining moiety A to base B may include any of the well known bonds including carbon-carbon single bonds, or carbon-oxygen single bonds. However, it is generally preferred that the chemical linkage include an olefinic bond at the ∂-position relative to B. The presence of such an ∂-olefinic bond serves to hold the moiety A away from the base when the base is paired with another in the well known

double-helix configuration. This permits interaction with polypeptide to occur more readily, thereby facilitating complex formation. Moreover, single bonds with greater rotational freedom may not always hold the moiety sufficiently apart from the helix to permit recognition by and complex formation with polypeptide.
Page 96, 1st ¶ ("By way of summary . . . The chemical moiety Sig so attached to the nucleotide P-S-B is capable of rendering or making the resulting nucleotide, now comprising P-S-B with the Sig moiety being attached to one or more of the other moieties, self-detecting or signalling itself or capable of making its presence known per se, when incorporated into a polynucleotide, especially a double-stranded polynucleotide. . . The Sig moiety desirably should not interfere with the capability of the nucleotide to form a double-stranded polynucleotide containing the special Sig-containing nucleotide . . . and, when so incorporated therein, the Sig-containing nucleotide is capable of detection, localization or observation.
Page 97, 2nd ¶ (" . . . the Sig component could comprise any chemical moiety which is attachable either directly or through a chemical linkage or linker arm to the nucleotide, such as to the base B component therein, or the sugar S component therein, or the phosphoric acid P component thereof.)

According to Dr. Donegan's opinion and conclusion at the end of Paragraph 14, the specification reasonably conveys that the inventors were in possession of the specific linkage group subject matter of claims 339-341, 350-351, 353-354 and 356-358 at the time of their 1982 application filing.

[2] As set forth in Paragraph 14 of his Declaration (Exhibit A), it is Dr. Donegan's opinion and conclusion that the specification supports the subject matter of claims 346 and 363 with respect to template dependent and template independent incorporation of modified nucleotides into oligo- or polynucleotides. As stated by Dr. Donegan, it was known in the art that the incorporation of modified nucleotides, or for that matter, any nucleotide, can be carried out under template dependent or template independent reactions and conditions. Dr. Donegan then lists in his Declaration the following several examples of template dependent incorporation and template independent incorporation reactions and methods, including many citations in the specification:

TEMPLATE DEPENDENT INCORPORATION

Example	Citation in Specification
nick translation	**Page 31, 1st full ¶** ("Modified nucleotides may be used in a method of gene mapping by *in situ* hybridization which circumvents the use of radioisotopes. This procedure takes advantage of a thymidine analogue containing biotin that can be incorporated enzymatically into DNA probes by nick translation.") **Page 32, 2nd ¶** (DNA probes were nick translated in the presence of Bio-dUTP . . .") **Page 67, Example XX** ("DNA was labeled with 5-substituted pyrimidine triphosphate by nick translating DNA in the presence of the appropriate triphosphate. . .") **Page 69, Example XXII** ("Phage T4 DNA and phage DNA were labeled by incorporation of H3-deoxyadenosine triphosphate into the DNA by nick translation . . .") **Add Page 70, last ¶** (". . . As previously indicated herein, nick translation is only one of a number of techniques and approaches possible for the production of the modified nucleic acids in accordance with this invention.") **Page 71, Example XXIII** ("Lambda DNA was nick translated . . .")
DNA polymerase	**Page 25, 1st ¶** ("These compounds can be made by enzymatic polymerization of appropriate nucleotides, especially nucleotide triphosphates in the presence of a nucleic acid template which directs synthesis under suitable conditions. . Illustrative enzymes include DNA polymerase I of E. coli, bacteriophage T4 DNA polymerase, DNA polymerases ∂ and ß from murine and human (HeLa) cells, DNA polymerase from Herpes simplex virus, . . ."
RNA polymerase	**Page 1, 6th & 7th lines from bottom** ("The biotin-labeled nucleotides are efficient substrates for a variety of DNA and RNA polymerases in vitro.) **Page 19, last five lines, through Page 20, line 9** ("Although biotinyl ribonucleoside triphosphates were found to function as substrates for the RNA polymerases . . . biotin-labeled RNA probes can be prepared enzymatically from DNA templates using E. coli or T7 RNA polymerases . . .") **Page 25, lines 10-13** (". . . RNA polymerase of E. coli, RNA polymerase of bacteriophage T7, eukaryotic RNA polymerase including HeLa cell RNA polymerase III, calf thymus polymerase II, and mouse cell RNA polymerase II.")

TEMPLATE INDEPENDENT INCORPORATION

Example	Citation in Specification
terminal transferase	**Page 56, Example III** ("Oligodeoxyribonucleotides were end-labeled using cytidine-5'-triphosphate and terminal transferase . . .") **Page 99, 2nd ¶** ("As indicated hereinabove, various techniques may be employed in the practices of this invention for the incorporation of the special nucleotides of this invention into DNA and related structures. One particularly useful technique . . . involves the utilization of terminal transferase for the addition of biotinated dUMP onto the 3' ends of a polypyrimidine or to single-stranded DNA. . .") **Page 100, 2nd ¶** ("Illustrative of the practices of this invention, biotinated dUTP was added to the 3' ends of d[pT]4 as well as single and double stranded DNA employing terminal transferase. . . The results . . . established that terminal transferase added biotinated dUMP to the 3' ends of a polypyrimidine.")
DNA ligation	**Page 77, Example XXXIV** ("A DNA probe was ligated to a synthetic DNA composed of repeated sequences of E. coli lac operator DNA. . .") **Page 60, Example IX** ("Ligation of poly dA:poly dT, biotinyl dU to oligodeoxyribonucleotides was accomplished as follows: . . .")
RNA ligation	**Page 20, lines 6-14** (". . biotin-labeled RNA probes can be prepared enzymatically . . . by 3' end-labelling methods using RNA ligase with compounds such as biotinyl-pCp. The AA- and NAGE-derivatives of UTP are, however, substrates for the eukaryotic RNA polymerases mentioned above.") **Page 25, 2nd ¶** (" . . . Moreover, the compounds such as pCp or pUp in which the base is biotinized can be added to existing molecules employing the enzyme RNA ligase.")
chemical modification	**Page 6, 1st full ¶** (" . . . Alternatively, nucleotides present in oligo- or polynucleotides may be modified using chemical methods.") **Page 25, 3rd ¶** ("Modified oligo- and polynucleotides can also be prepared by chemical modification of existing oligo- or polynucleotides using the approach described previously for modification of individual nucleotides.")

In concluding Paragraph 14 in his Declaration, Dr. Donegan indicates that a reading of the specification reasonably conveys that the present inventors were in possession of the template dependent and template independent incorporation subject matter of claims 346 and 363 at the time this application was originally filed in 1982.

[3] In Paragraph 15 and Subparagraphs A-C of his Declaration (Exhibit A), Dr. Donegan states that it is his opinion and conclusion that the specification supports the of claims 365-367 with respect to the specific localization of modified nucleotides in an oligo- or polynucleotide. In Subparagraph A, Dr. Donegan points out that the chemical structure on page 23 clearly shows an oligo- or polynucleotide having an internal modified nucleotide as recited in claim 365. Dr. Donegan also notes that on page 24, penultimate paragraph, the specification discloses that more than one modified nucleotide may be incorporated:

> It is also to be understood that the structure embraces more than one modified nucleotide present in the oligomer or polymer, for example, from two to thirty modified nucleotides. . . ."

Further, on page 27, second paragraph, the specification discloses:

> . . . For example, pBR 322 DNA or λ DNA, nick translated to introduce approximately 10-100 biotin residues per kilobase, . . .

Moreover, according to Dr. Donegan, the specification is replete with references and examples directed to the incorporation of modified nucleotides by nick translation. Dr. Donegan points out that nick translation would result in oligos- or polynucleotides each containing multiple modified nucleotides, citing Page 67 (Example XX), Page 69 (Example XXII) and Page 71 (Example XXIII), in the specification.

According to Dr. Donegan, external modified nucleotides as set forth in claim 366 are disclosed in the specification by virtue of the numerous instances where terminal transferase is disclosed or employed to add modified nucleotides to the 3′ end of an oligo- or polynucleotide. Reference is given in his Declaration to

page 56 (Example III), page 99, 2nd paragraph, and page 100, 2nd paragraph.

It is also Dr. Donegan's opinion and conclusion that the subject matter of claim 367 ("labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one internal modified nucleotide and at least one external modified nucleotide") is also supported by the specification. Dr. Donegan notes that as indicated in Paragraph 14 of his Declaration, means for incorporating modified nucleotides into oligo- and polynucleotides internally, such as by nick translation, and externally (terminally), such as by terminal transferase, are disclosed numerous times in the specification. Further, he notes that as also indicated in the preceding Subparagraph 15A of his Declaration, the specification clearly discloses in the penultimate paragraph on page 24 that multiple modified nucleotides can be incorporated into an oligomer or polymer. According to Dr. Donegan, these several disclosures in the specification, taken with the chemical formula on page 23 in which m or n can be zero, forms the basis of his opinion and conclusion that the subject matter of claim 367 is supported.

The next two issues in the new matter rejection are addressed in reverse order to follow the order in Dr. Donegan's Declaration.

Sequencing Gel Practice

[5] As set forth in Paragraph 7 of his Declaration (Exhibit A), Dr. Donegan declares that it is his opinion and conclusion that the sequencing gel practice of claims 329 and 348 is supported by the disclosure in the specification at page 84, second paragraph:

> This type of self-signaling molecule can be used to monitor any nucleic acid hybridization reaction. It is particularly important for detecting nucleic acids in gels (for example, **sequencing gels**).
> [bold added]

The information in Paragraphs 8-11 of Dr. Donegan's Declaration formed the basis for his opinion and conclusion that the specification reasonably conveys that the inventors were in possession of the sequencing gel practice of claims 329 and 348 at the time the application was filed in 1982.

In Paragraph 8 of his Declaration, Dr. Donegan notes that sequencing gels and their use have been long accepted in the art, including at the time this application was originally filed in 1982. According to Dr. Donegan, the very term "sequencing gel" and equivalent terminology such as gel sequencing of nucleic acids, DNA sequencing gels and nucleic acid sequencing gels, were well known and accepted in the art before 1982. Dr. Donegan notes that the two classic papers on nucleic acid sequencing were published by Maxam and Gilbert, "A new method for sequencing DNA," Proc. Natl. Acad. Sci. (USA) 74:560-564 (February 1977); and Sanger et al., "DNA sequencing with chain-terminating inhibitors," Proc. Natl. Acad. Sci. (USA) 74:5463-5467 (December 1977). Dr. Donegan relates that even before the 1977 publication of the now legendary papers by the Gilbert and Sanger groups, other more tedious methods for sequencing were available, including, for example, Professor Sanger's own "plus and minus" method and "ribosubstitution," the latter a method developed by Wayne M. Barnes, Dr. Sanger's own post-doctoral fellow working in the Sanger laboratory.

Dr. Donegan points out that Professor Gilbert himself employed the term "sequencing gel" in his famous 1977 PNAS paper on page 563, right column, and continuing through page 564, left column:

> **Gel Samples.** All samples for *sequencing gels* are in 10 or 20 μl of 0.1 M NaOH/1 mM EDTA to which is added an equal volume of 10 M urea/0.65% xylene cyanol/0.05% bromphenol blue. Heat the sample at 90° for 15 sec, the layer on the gel.
>
> ***Sequencing Gels.*** These are commonly slabs 1.5 mm X 330 mm X 400 mm with 18 sample wells 10 mm deep and 13 mm wide separated by 3 mm (fitting on a 35.5 X 43 cm x-ray film). They are 20% (wt/vol) acrylamide (Bio-Rad)0.67% (wt/vol) methylene bisacrylamide/7 M urea/50 mM Tris-borate, pH 8.3/1 mM EDTA/3 mM ammonium persulfate, 300 ml of gel solution is polymerized with TEMED within 30 min (generally 50 μl of TEMED). Age the gel at least 10 hr before using it. Electrophorese with some heating (30-40°), to help keep the DNA denatured, between 800 and 1200 V. Load successively whenever the previous xylene cyanol has moved halfway down the gel. Bromphenol blue runs with 10-nucleotide-long fragments, xylene cyanol with 28. With three loadings at 0, 12, and 24 hr, a 1000-V run for 36 hr permits reading more than 100 bases. To sequence the first few bases from the labeled end, use a 25% acrylamide/0.83% bisacrylamide gel in the usual urea buffer and pre-electrophorese this gel for 2 hr at 1000 V.

Autoradiography. Freeze the gel for autoradiography. . .
[bold in original, italic & underline added]

According to Dr. Donegan, several other authors and investigators quickly followed suit by publishing articles that also used these terms. These include:

Dr. Barnes ["DNA Sequencing by Partial Ribosubstitution," Journal of Molecular Biology 119:83-99 (1978)];

Winter and Brownlee ["3' End labelling of RNA with ^{32}P suitable for rapid gel sequencing" [Nucleic Acids Research 5:3129-3138 (1978];

Maxam and Gilbert, "Sequencing End-Labeled DNA with Base-Specific Chemical Cleavages," Methods in Enzymology, Volume 65, Part I, Lawrence Grossman and Kivie Moldave, editors, Academic Press, New York, 1980, pages 497-701];

Andrew Smith ["DNA Sequence Analysis by Primed Synthesis," pages 560-580 in the same volume of Methods in Enzymology as Maxam and Gilbert's 1980 techniques paper;

Ambartsumyan and Mazo, "Elimination of the Secondary Structure Effect in Gel Sequencing of Nucleic Acids" , FEBS Letters 114:265-268 (1980);

Garoff and Ansorge, "Improvements of DNA Sequencing Gels," Analytical Biochemistry 115:450-457 (1981);

Gingeras et al. "A semi-automated method for the reading of nucleic acid sequencing gels," Nucleic Acids Research 10:103-114 (1982); and

Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 1982, Appendix A, Protocol for Sequencing by the Maxam-Gilbert Technique, page 475;

In Paragraph 10 of his Declaration, Dr. Donegan notes that even after the application's filing in 1982, the term "sequencing gel" continues to be well-accepted in the art even to this very year. Dr. Donegan offers several scientific and technical dictionaries for the term "sequencing gel" including:

A Dictionary of Genetic Engineering, Oliver and Ward, Cambridge University Press, Cambridge, 1985, page 100:

> **sequencing gel** A long polyacrylamide slab gel which has sufficient resolving power to separate single-stranded fragments of DNA or RNA which differ in length by only

> a single nucleotide. Electrophoresis is carried out at high voltage and with the gel in a vertical position. Urea is usually included in the gel mixture as a denaturing agent. This prevents internal base pairing within the single-stranded molecules and ensures that their relative speed of migration is solely dependent on their length. Such gels are used to separate the radioactively labelled products of, for example, the Maxam-Gilbert or the Sanger sequencing reactions.

Dictionary of Biochemistry and Molecular Biology, 2nd Edition, J. Stenesh, John Wiley & Sons, New York, 1989, page 437:

> **sequencing gel** A long, thin polyacrylamide gel slab used for nucleic acid sequencing.

Oxford Dictionary of Biochemistry and Molecular Biology, Smith et al., editors, Oxford University Press, New York, 1997, page 594:

> **sequencing gel** a polyacrylamide gel run to resolve oligonucleotides produced in a DNA sequencing procedure. *See* **chain-termination method, chemical cleavage method.** [bold & italic in original]

Dictionary of Plant Genetics and Molecular Biology, G. Miglani, The Food Products Press, New York, 1998, page 258:

> **sequencing gel:** A long, polyacrylamide salt gel that has sufficient resolving power to separate single-stranded fragments of DNA or RNA which differ in length by only a single nucleotide. Electrophoresis is carried out at high voltage and with the gel in a vertical position.

Finally, in Paragraph 11 of his Declaration, Dr. Donegan notes that the term "sequencing gel" was also used quite liberally throughout a textbook that was devoted entirely to the topic of DNA sequencing and that was published the year after this application. In the book titled DNA Sequencing, [Elsevier, Amsterdam and New York, 1983, 384 pages], published as part of the series Laboratory Techniques in Biochemistry and Molecular Biology, the author Hindley refers to the term "sequencing gel" no less than fifty-one (51) times on forty-five (45) different pages.

Based upon the foregoing publications all of which are cited in his Declaration, it is Dr. Donegan's opinion and conclusion that the use of the term "sequencing gels" in the specification of this application (page 84, second

Principle of the Method. Atkinson *et al.* (4) showed that the inhibitory activity of 2',3'-dideoxythymidine triphosphate (ddTTP) on DNA polymerase I depends on its being incorporated into the growing oligonucleotide chain in the place of thymidylic acid (dT). Because the ddT contains no 3'-hydroxyl group, the chain cannot be extended further, so that termination occurs specifically at positions where dT should be incorporated. If a primer and template are incubated with DNA polymerase in the presence of a mixture of ddTTP and dTTP, as well as the other three deoxyribonucleoside triphosphates (one of which is labeled with ^{32}P), a mixture of fragments all having the same 5' and with ddT residues at the 3' ends is obtained. When this mixture is fractionated by **electrophoresis** on denaturing acrylamide gels the pattern of bands shows the distribution of dTs in the newly synthesized DNA. By using analogous terminators for the other nucleotides in separate incubations and running the samples in parallel on the gel, a pattern of bands is obtained from which the sequence can be read off as in the other rapid techniques mentioned above.

Sanger et al. (1977), page 5463, left column, penultimate ¶, [bold added]

Barnes' 1978 JMB paper:

. . . A key observation made by Sanger and his colleagues was that **electrophoresis on acrylamide gels** can resolve DNA molecules differing in length by a single nucleotide over the range of 20 to 140 nucleotides, if the molecules to be analysed all have the same sequence and share the same 5' end (Barrell *et al.* 1976). Maxam and Gilbert (1977) use **similar high-resolution acrylamide gels** in a DNA sequencing method that uses the principle of end-labelling and base-specific partial chemical cleavage. . .

Barnes (1978), page 83, [bold added]

Winter and Brownlee (1978):

(3) **Sequencing gel**. The conditions are essentially as described in Simonosits et al. [1] but were **adapted for use with thin gels** [18]. 1 µl aliquots of labelled tRNA were dried and digested in 2 µl as follows: 0.003 units RNase T_1, 0.1 M Tris-HCl, 10 mM EDTA pH 7.5, 0°C, 2 min and 10 min; 2 pg RNase A, pH 7.5 buffer (as for T_1 RNase), 0°C, 5 min and 30 min; 0.4 units RNase U_2, 8.75 M urea, 20 mM sodium acetate, 2 mM EDTA, 50°C, 5 min; 0.0014 units RNase Phyl, 10 mM sodium acetate, 1 mM EDTA, pH 5.9, room temperature, 1½ min and 20 min; formamide containing 1 µl 1 M magnesium acetate per ml, 100°C, 30 min. For the ladder, traces of magnesium ion were added to the hot formamide to catalyse the degradation (R.H. Symons and G.P. Winter, unpublished). Partial alkali cleavage could have been used for the ladder since the mixture

of cyclic phosphate and 2′ and 3′ phosphates so generated are not attached to the radioactively labelled fragments. With 5′ labelled sequences, however, the different mobilities of small oligonucleotides with cyclic and open phosphates introduces extra bands into the early portions of the ladder [1]. Time points were combined before running and a control of undigested $tRNA^{Phe}$ was included. Reactions were stopped by adding 2 µl of formamide dye mixture and heating at 100°C for 1 min. 2 µl of **sample were applied to the thin gel which was electrophoresed** at 1.6 kV for 4 h; the remaining 2 µl was applied after reheating and the **gel electrophoresed** for a further 2 h. The gel was exposed to preflashed film for five days [20]. [bold added]

Maxam and Gilbert's 1980 Methods in Enzymology techniques paper:

Gel sequencing methods have traditionally employed versions of a pH 8.3 polyacrylamide gel described by Peacock and Dingman[62] and adapted for small single-stranded DNA molecules by Maniatis *et al.*[63]
Page 540, first full paragraph

Garoff and Ansorge (1981):

The new and rapid DNA sequencing techniques involve the generation of a set of oligonucleotides, which have one end in common and the other end varying in length with a single nucleotide, and the subsequent separation of the oligonucleotides on denaturing polyacrylamide gels. page 430, right column, 1st ¶

A Dictionary of Genetic Engineering, Oliver and Ward, page 100:

> **sequencing gel** A long polyacrylamide slab gel which has sufficient resolving power to separate single-stranded fragments of DNA or RNA which differ in length by only a single nucleotide. **Electrophoresis** is carried out at high voltage and with the gel in a vertical position. Urea is usually included in the gel mixture as a denaturing agent. This prevents internal base pairing within the single-stranded molecules and ensures that their relative speed of migration is solely dependent on their length. Such gels are used to separate the radioactively labelled products of, for example, the Maxam-Gilbert or the Sanger sequencing reactions.
> [bold added]

Dictionary of Biochemistry and Molecular Biology, Stenesh, page 437:

> **sequencing gel** A long, thin polyacrylamide gel slab used for nucleic acid sequencing.

Oxford Dictionary of Biochemistry and Molecular Biology, Smith et al., editors, page 594:

> **sequencing gel** a polyacrylamide gel run to resolve oligonucleotides produced in a DNA sequencing procedure. *See* **chain-termination method, chemical cleavage method.** [bold & italic in original]

Dictionary of Plant Genetics and Molecular Biology, G. Miglani, page 258:

> **sequencing gel:** A long, polyacrylamide salt gel that has sufficient resolving power to separate single-stranded fragments of DNA or RNA which differ in length by only a single nucleotide. **Electrophoresis** is carried out at high voltage and with the gel in a vertical position.
>
> [bold added]

Hindley's book of DNA Sequencing, "Preliminary Remarks" in the Introduction (Chapter 1):

> 1.1 Preliminary Remarks
> The present art of DNA sequencing has its origins in a variety of different fields of nucleic acid enzymology and chemistry. Indeed as early as 1970 our knowledge and understanding of these fields was, in theory, sufficiently far advanced to anticipate the development of the modern rapid methods but two obstacles had first to be overcome to convert these ideas into reality. The first was the problem of separating the oligonucleotides, generated in the sequencing reactions, in a rapid convenient and reproducible manner and displaying them as an ordered set of fragments according to their chain length. While the technique of homochromatography, in which a random mixture of polynucleotides of all possible chain lengths is used to develop a chromatogram (Brownlee and Sanger, 1969), was an important step in this direction, it was through the development of gel electrophoretic techniques that this problem was finally solved. **All the methods to be described rely on the extraordinary resolving power of polyacrylamide gels** run under denaturing conditions to achieve the final separations; much effort has gone into perfecting such systems so as to optimise their resolving properties. . ."
>
> [Hindley, DNA Sequencing, page 1, bold added]

Thus, according to Dr. Donegan and based upon the documents referenced in Subparagraphs 16A-16H of his Declaration, and further based upon his own experience and knowledge at the time this application was filed in 1982, it would have been understood that the practice and use of sequencing gels intrinsically

involved electrophoretic separation as set forth in claim 368.

[6] Applicants respectfully point out that it was not until the advent of their present invention had anyone attempted to label nucleotide bases in the disruptive and semi-disruptive positions, the so-called "non-Ward positions." In order to understand the basis of the present invention, it would be helpful to describe briefly the state of technology with respect to nucleic acid labeling and detection in the early 1980s. In 1981, Dr. David C. Ward and his group at Yale became textbook celebs for their discovery that nucleotides could be non-radioactively labeled in the so-called non-disruptive positions of the base without substantially interfering with the capability of the labeled nucleotide to be incorporated into an oligo- or polynucleotide, and without substantially interfering with the capability of the oligo- or polynucleotide to be detected by means of the labeled nucleotide that was incorporated. Prior to 1981, nucleic acids were conventionally labeled with radioactive isotopes, most notably ^{32}P. With Dr. Ward's discovery, the world turned en masse to non-radioactive labeling of nucleic acids, that discovery culminating in the issuance of several United States and foreign patents including the following: U.S. Patent Nos. 4,711,955; 5,328,824; 5,449,767; 5,476,928; and European Patent Nos. 0 063 879 B1 and 0 329 198 B1. Dr. Ward himself has been recognized for his achievements by his recent election to the National Academy of Science.

The principles or criteria behind the Ward discovery are exquisitely set forth in their patent specifications. In U.S. Patent No. 5,328,824, for example, the Ward "criteria" for base labeling nucleotides are described in columns 6 and 7 under the section titled "DETAILED DESCRIPTION OF THE INVENTION:'

> Several essential criteria must be satisfied in order for a modified nucleotide to be generally suitable as a substitute for a radioactively-labeled form of a naturally occurring nucleotide. First, the modified compound must contain a substituent or probe that is unique, i.e., not normally found associated with nucleotides or polynucleotides. Second, the probe must react specifically with chemical or biological reagents to provide a sensitive detection system. Third, the analogs must be relatively efficient substrates for commonly studied nucleic acid enzymes, since numerous practical applications require that the analog be enzymatically metabolized, e.g., the analogs must function as substrates for nucleic acid

polymerases. For this purpose, probe moieties should not be placed on ring positions that sterically, or otherwise, interfere with the normal Watson-Crick hydrogen bonding potential of the bases. Otherwise, the substituents will yield compounds that are inactive as polymerase substrates. Substitution at ring positions that alter the normal "anti" nucleoside conformation also must be avoided since such conformational changes usually render nucleotide derivatives unacceptable as polymerase substrates. Normally, such considerations limit substitution positions to the 5-position of a pyrimidine and the 7-position of a purine or a 7-deazapurine.

Fourth, the detection system should be capable of interacting with probe substituents incorporated into both single-stranded and double-stranded polynucleotides in order to be compatible with nucleic acid hybridization methodologies. To satisfy this criterion, it is preferable that the probe moiety be attached to the purine or pyrimidine through a chemical linkage or "linker arm" so that it can readily interact with antibodies, other detector proteins, or chemical reagents.

Fifth, the physical and biochemical properties of polynucleotides containing small numbers of probe substituents should not be significantly altered so that current procedures using radioactive hybridization probes need not be extensively modified. This criterion must be satisfied whether the probe is introduced by enzymatic or direct chemical means.

Finally, the linkage that attaches the probe moiety should withstand all experimental conditions to which normal nucleotides and polynucleotides are routinely subjected, e.g., extended hybridization times at elevated temperatures, phenol and organic solvent extraction, electrophoresis, etc.

All of these criteria are satisfied by the modified nucleotides described herein.

In their present invention, Applicants achieved the unthinkable: nucleic acid labeling in the disruptive and semi-disruptive positions of the sugar, phosphate and base moieties without significantly affecting the capability of oligo- and polynucleotides containing such modified labeled nucleotides from participating in hybridization reactions. Having reached the present invention in contravention of certain Ward teachings, it seems unfair to deny Applicants' the full scope of coverage by limiting their claims as set forth in the rejection at hand.

Applicants expect to submit further information with respect to this issue in the very near future.

In view of the Donegan Declaration and its attached exhibits and the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the new matter rejection.

The Rejection Under 35 U.S.C. § 112, First Paragraph

Claims 284-372 stand rejected under 35 U.S.C. § 112, first paragraph, as the disclosure is allegedly enabling only for claims limited to a scope of covalent attachment sites of the cited "Sig" moiety to bases of nucleic acids wherein said sites are either the N^2 of guanine, the N^6 of cytosine, or the C^6 of uracil. In the Office Action (pages 5-8), the Examiner stated:

> [1] A thorough review of the disclosure as filed has revealed that the chemistry by which nucleic acid bases may be modified so as to attach a "Sig" moiety only is disclosed for the above four attachment sites within the scope of claims 284 etc. For example, the instant disclosure does not discuss in any way the preparation of N-1 or N-3 modified purines or N-3 or C-2 modified pyrimidines. It is noted that claims 284 etc. are already limited in that certain other, non-base, attachment sites on purines, pyrimidines, and deazapurines are not within the scope of the claims for the at least one modified base in probes used in the claimed methods. It is also noted that certain generalized labeling methods are instantly disclosed such as the formaldehyde coupling of cytochrome C as a bridge between biotin and a nucleic acid molecule on page 58 but that such generalized labeling of a nucleic acid probe lacks both instant disclosure as well as predictability as to where the attachment site is on the probe and therefore fails to predictably form attachments as instantly claimed and thus is deemed to fail to enable the broad scope of specific base modifications of the instant claims. Ruth is herein cited as summarizing the lack of knowledge at the time of the instant filing regarding the preparation of nucleic acid hybridization probes which contain a signalling moiety. The earliest disclosure of said summary of Ruth is 2/22/83 which is the filing date of the earliest patent thereof and which is also less than a year after the filing date of the instant application. This therefore summarizes the lack of broad hybridization probe preparatory knowledge even after the instant filing date. Ruth summarizes the preparatory knowledge for signal moiety containing labeled probes in column 1, line 43, through column 3, line 45. As cited therein nucleic acid hybridization probes may be prepared either chemically or enzymatically. Enzymatic synthesis using nick translation is discussed wherein certain base modifications have been incorporated into probes but limited in use due to several factors. One of these factors is that only certain

modifications may be incorporated by enzymes. Ward et al. (P/N 4,711,955) summarize the factors that were viewed as limitations on modified nucleotides in column 6, line 36, through column 7, line 17, and thereinafter discuss specific base modifications with detailed and lengthy chemical steps. Ruth at column 3, lines 26-45, also summarizes that chemical synthesis has not been disclosed in the prior art as incorporating modified or reporter group containing nucleotides. Further consideration of Ruth reveals that specific base modifications are therein disclosed such as at column 10, line 57, through column 20 which are accomplished via a lengthy series of detailed reactions including the masking and unmasking of reactive side groups to prevent unwanted modifications. Ruth and Ward et al. are deemed representative of those skilled in the art at about the time of the instant filing date of the instant disclosure. In summary, those skilled in the art at the time of filing of the instant invention viewed the preparation of signal moiety containing nucleic acid probes as lengthy and detailed procedures that were discussed as being accomplished only for certain specific base modifications. It is noted that Ruth or Ward et al. only disclose base modifications at the following sites: C-8 of purines and the C-5 of pyrimidines, N^6 of adenosine, and N^2 of guanosine, and N^4 of cytosine, and C-7 of 7-deazapurines. This documents the lack of enablement of most specific base modifications without detailing lengthy preparatory procedures for those skilled in the art at the time of the instant filing date. Therefore it is deemed undue experimentation to prepare base modified nucleic acid hybridization probes wherein the site of base modifications is other than N^2 of guanine, the N^6 of adenine, the N^4 of cytosine, or the C-6 of uracil within the scope of instant claims 240 etc. It is again noted that the instant claims are limited so that base modifications at the C-8 of purines, the C-5 of pyrimidines, and the C-7 of 7-deazapurines are not within their scope. This rejection is reiterated and newly applied as necessitated by amendment due to newly added claims. The rejection has not been argued on its merits other than pointing to issued patents.

[2] Claims 284-372 are rejected under 35 U.S.C. § 112, first paragraph, as the disclosure is enabling only for claims limited to "SM" moieties which are either ribose or deoxyribose. It is noted that claim 284, lines 13-15, cite "PM" attachment points when the nucleotide compound is either a deoxyribonucleic or ribonucleotide but does not therein limit the "SM" moiety to a sugar moiety that is present in either of these nucleotide types. Thus, the scope of "SM" is only presently limited in claims 284 etc. to being a "sugar moiety" which is much broader in scope than that of ribose or deoxyribose. It is noted that there is no instant discussion as to how to practice the synthesis of nucleotides with "SM" moieties other than that of ribose or deoxyribose. For example, how does someone wishing to utilize glucose as "SM" practice the instant claims? It is noted that in order to broadly practice sugar moieties usage both the synthesis of "PM" attachment is required as well as the "Sig" attachment. Additionally hybridization between the nucleic acid of interest and the oligo- or polynucleotide must still be permitted. No guidance whatsoever has been instantly set forth directed to accomplishing this broad sugar

> moiety practice other than that directed to ribose or deoxyribose sugars. It is noted additionally that the numerous examples given in the specification do not include any sugar practice other than ribose or deoxyribose. In the above scope rejection directed to base labeling practice the need for detailed and lengthy procedures to enable the person skilled in the art to prepare nucleotide analogs as well as their incorporation into polymers is summarized. These disclosures include complex chemical protection requirements including those directed to sugar side group protection as well as considerations such as whether enzymes would recognize and incorporate nucleotides into polymers or not as well as other considerations as discussed above. Thus, it is deemed undue experimentation to practice nucleotide compound and polymers containing these compounds without such detailed and lengthy procedural guidance. In summary, such detailed and lengthy guidance is instantly set forth only for "SM" practice directed to ribose or deoxyribose and it is deemed undue experimentation to practice "SM" moieties other than ribose and deoxyribose given the limited instant disclosure. This rejection is reiterated and newly applied as necessitated by amendment due to newly added claims. The rejection has not been argued on its merits other than pointing to issued patents.

The rejection for lack of enablement is respectfully traversed again.

[1] As indicated in the preceding rejection, Applicants expect to submit further information in due course relating to the issue of base labeling.

[2] With respect to the sugar issue, as noted above in the opening remarks of this Amendment, each of the independent claims has been amended to recite a "monosaccharide" moiety instead of a "sugar moiety." It is believed that the monosaccharide recitation represents a proper scope of claim coverage in light of Applicants' disclosure. In view of the above amendments to the claims, reconsideration and withdrawal of the second issue in the instant rejection is respectfully requested.

The Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 284-372 stand rejected for indefiniteness under 35 U.S.C. § 112, second paragraph. In the Office Action (pages 10-13), the Examiner stated:

[1] Claims 337-372 are vague and indefinite because duplicate and confusing step notations are present. For example, claim 337, lines 4 and 14 both cited step (l). Also, claim 337, third from the last line, confusingly cites step (b) without any corresponding step (a). Claim 348, last line of step (B), cites a (b) without a corresponding (a). Also, this (b) is the second (b) because there is a (b) in line 20. These rejections are necessitated by amendment.

[2] Claim 284, part (b), cites the detection of the presence of "oligo- or polynucleotides which have hybridized to said nucleic acid of interest" but is vague and indefinite when considered in view of part (a) of the claim. Said part (a) cites the practice of "hybridizing..." without any selectivity or specificity directed to preventing hybridization to nucleic acids that are not the "nucleic acid of interest". Thus, such "permitting" practice is reasonably interpreted as inclusive of all levels of stringency including conditions where hybridization is permitted to not only "nucleic acid of interest" but also to other nucleic acids that may be only 90% complementary, 70% complementary, or even only 20% complementary, etc. to the "oligo- or polynucleotide" cited in part (a). With this broad complementarity practice possible within the scope of part (a), what is meant by applicants' citation of the detecting practice of part (b)? Do applicants mean that selectivity or specificity is to be practiced at the detection step and not at the hybridization step? This suggests that the detecting step is not just a detecting step but is also inclusive of some selection practice. Such a selection practice is not given in step (b) as presently worded. It is noted that the commonly performed practice of a hybridization assay is to control the hybridization step, herein step (a) rather than step (b), so as to be selective as desired. Then the detection step is only directed to the detection of a signal which is then indicative of the presence of the "nucleic acid of interest" in the sample. This, however, is not how claim 284 is presently worded. This unclarity causes even more concern regarding claims such as 324 or 325 which are directed to genetic disorder detection. Additionally there is no mention of the "Sig" moiety in the detection practice of step (b) whereas it is the only "detectable" moiety that is cited in part (a). Do applicants intend that the detection practice of part (b) is inclusive of detection without use of the "Sig" moiety from part (a)? Alternatively, if detection of the "Sig" moiety of part (a) is intended to be the manner of detection of hybridization in part (b), why is part (b) silent regarding said "Sig" moiety? Clarification is requested as to what applicants mean for the metes and bounds of parts (a) and (b) regarding how the presence of the "nucleic acid of interest" is indicated in the sample versus nucleic acids that are not of interest and what signal is determinative of said presence. Do applicants mean to include some selectivity in either of parts (a) or (b) and, if so, which part or parts? This unclarity is present in all of the instantly depending claims due to their direct or indirect dependence from any of the instant independent claims. This rejection is reiterated and newly applied as necessitated by amendment due to newly added claims.

> **[3]** All of the instant independent claims and those dependent therefrom directly or indirectly all are vague and indefinite because the metes and bounds of the positions on the base at which the Sig moiety is covalently attached is not commensurate with the various disclosures in the specification. See, for example, the directive on page 53, lines 1-4, which limits the modifications as to not interfering with the formation of a double-helix which is not recited in the claims. This rejection is reiterated and newly applied as necessitated by amendment due to newly added claims.
>
> **[4]** Claims 329-336 and 348-372 are vague and indefinite as to what is meant by "self-signalling", "self-indicating", or "self-detecting" because signals in assays must be received outside of the reaction moieties in order to record the reaction event. What, therefore, is meant by "self-..." which suggests the signal being turned in unto itself? Clarification is requested. This rejection is necessitated by amendment.

The indefiniteness rejection is respectfully traversed.

The remarks which follow below are directed to the bold bracketed numbers which have been inserted in the passage quoted from the January 6, 1998 Office Action.

[1][2] With respect to these matters raised in the indefiniteness rejection, Applicants believe that the amendments effected by their July 6, 1998 Amendment obviate the grounds for the rejection of claims 284, 337 and 348.

[3] As noted in their opening remarks, Applicants have adopted amended each of claims 284, 331, 337 and 348 by inserting language to the effect that the covalent attachment of Sig "does not substantially interfere with double helix formation." As noted by the Examiner in the instant Office Action, support for this language is taken from the specification, page 53, lines 1-4.

[4] With respect to the phrase "self-signaling or self-indicating or self-detecting" in various claims (329, 332 and 348), Applicants respectfully maintain that this language is altogether proper and passes the statutory strictures for definiteness. It is believed that a reader skilled in the art would readily comprehend the meaning of modified nucleotides that are self-signaling or self-indicating or self-detecting, particularly in light of the specification and the knowledge in the art. It is generally understood that self-signaling or self-

indicating or self-detecting modified nucleotides and oligo- or polynucleotides containing them provide a means for direct detection including fluorescence, chemiluminescence and chelation. Applicants are mindful that certain dependent claims are directed to embodiments for such "self-signaling or self-indicating or self-detecting" modified nucleotides. For example, claim 333 that depends from claim 329, recites "wherein said self-signalling or self-detecting modified nucleotide comprises a member selected from the group consisting of a fluorescent component, a chemiluminescent component, and a chelating component, or a combination of any of the foregoing." Claims 370 and 371 depend ultimately from claim 348. The former recites "wherein said direct detection is carried out on one or more self-signaling or self-indicating or self-detecting nucleotides." The latter recites "wherein said one or more self-signaling or self-indicating or self-detecting nucleotides comprise fluoresceinated nucleotides." Another claim 372 that depends from claim 371, recites "wherein said fluoresceinated nucleotides comprise fluoresceinated DNA." Based upon a reading of the claim language in light of the specification, the language in other dependent claims and the knowledge in the art, the reader who would also be skilled in the art would understand the metes and bounds posed by modified nucleotides that are "self-signaling or self-indicating or self-detecting."

In addition, Applicants would like to draw attention to U.S. Patent Nos. 4,649,121 and 5,233,044, both of which recite "self-indicating" in various issued claims. See, for example, claims 6 and 7 in the '121 Patent, and claim 1 in the '044 Patent. A copy of the aforementioned two patents are attached as Exhibits C and D, respectively.

In view of the amendments to the claims and the foregoing remarks and submitted exhibits, Applicants respectfully request reconsideration and withdrawal of the indefiniteness rejection.

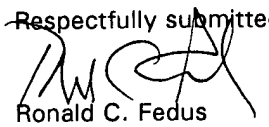
SUMMARY AND CONCLUSIONS

Claims 284-372 are presented for further examination on the merits. Claims 284, 331, 332, 337 and 348 have been amended. No other claims have been amended, canceled or added by this Amendment.

No fee is deemed necessary in connection with the filing of this Supplemental Response, fees having been previously authorized in Applicants' July 6, 1998 Amendment Under 37 C.F.R. §1.116, both for Applicants' request for the withdrawal of the finality of the January 6, 1998 Office Action and their three month extension request. If any other fee or fees are deemed necessary, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 05-1135.

If a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney request that he be contacted at the number provided below.

Respectfully submitted,



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